NEUROPROTECTIVE EFFECTS OF KYNURENINE AND RELATED COMPOUNDS. ELECTROPHYSIOLOGICAL AND BEHAVIORAL STUDIES

Ph.D. Thesis

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Direct related list of publications

I. **Németh, H.**, Robotka, H., Kis, Z., Rózsa, É., Janáky, T., Somlai, C., Marosi, M., Farkas, T., Toldi, J. and Vécsei, L. (2004) Kynurenine administered together with probenecid markedly inhibits pentylenetetrazol-induced seizures. An electrophysiological and behavioral study. *Neuropharmacology* **47**: 916-925

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II. Füvesi, J., Somlai, C., **Németh, H.**, Varga, H., Kis, Z., Farkas, T., Károly, N., Dobszay, M., Penke, Z., Penke, B., Vécsei, L. and Toldi, J. (2004) Comparative study on effects of kynurenic acid and glucoseamine-kyurenic acid. *Pharmacology Biochemistry and Behavior* **77**: 95-102

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III. **Németh, H.**, Toldi, J. and Vécsei, L. (2005) Role of kynurenines in the central and peripherial nervous systems. *Current Neurovascular Research* **2**: 249-260

IV. Robotka, H., **Németh, H.**, Somlai, C., Vécsei, L. and Toldi, J. (2005) Systemically administered glucosamine-kynurenic acid, but not pure kynurenic acid, is effective in decreasing the evoked activity in area CA1 of the rat hippocampus. *European Journal of Pharmacology* **513**: 75-80

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V. **Németh, H.**, Varga, H., Farkas, T., Kis, Z., Vécsei, L., Horváth, S., Boda, K., Wolff, J. R. and Toldi, J. (2002) Long-term effects of neonatal MK-801 treatment on spatial learning and cortical plasticity in adult rats. *Psychopharmacology* **160**: 1-8

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I. **Németh, H.**, Somlai, C., Varga, H., Kis, Z., Farkas, T., Károly, N., Dobszay, M., Penke, B., Vécsei, L. and Toldi, J. The cortical effects of kynurenic acid and of glucoseamine-kynurenic acid: a complex behavioural and electrophysiological study.

9th Annual Meeting of the Hungarian Neuroscience Society, Balatonfüred, 22-25th January, 2003, Clinical Neuroscience **56**: 62

II. Robotka, H., Marosi, M., **Németh, H.**, Toldi, J. and Vécsei, L. Effect of kynurenine (IP) on pentylenetetrazol-induced seizure in the rat hippocampus. *IBRO International Workshop, Budapest, 29-31 January, 2004, Clinical Neuroscience* **57**: 57

III. Robotka, H., Marosi, M., **Németh, H.**, Lür, G., Somlai, C., Toldi, J. and Vécsei, L. Systemically administered glucosamine-kynurenic acid, but not pure kynurenic acid, is effective in decreasing the evoked activity in area CA1 of the rat hippocampus. *11th Annual Meeting of the Hungarian Neuroscience Society, Pécs, 25-29th January, 2005, Clinical Neuroscience* **58**:

IV. **Németh, H.**, Robotka, H., Marosi, M., Kis, Z., Farkas, T., Vécsei, L. and Toldi, J. Kynurenine administered together with probenecid markedly inhibits pentylenetetratol-induced seizures. An electrophysiological and behavioural study. *11th Annual Meeting of the Hungarian Neuroscience Society, Pécs, 25-29th January, 2005, Clinical Neuroscience* **58**:

V. **Németh, H.**, Farkas, T., Kis, Z., Vécsei, L., Imre, G. and Toldi, J. Long-term effects of neonatal MK-801 treatment on spatial learning and cortical plasticity. *IBRO International Workshop, Debrecen, 24-26th, 2002, Neurobiology* **9**: 344

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Abbreviations

3-HK	3-hydroxykynurenine
7-Cl-KYNA	7-chlorokynurenic acid
ANA	anthranilic acid
ATP	adenosine triphosphate
BBB	blood-brain barrier
CSF	cerebrospinal fluid
EAA	excitatory amino acid
GABA	γ-aminobutyric acid
G-KYNA	glucosamine conjugate of kynurenic acid
i.c.v.	intracerebroventricularly
IDO	indolamine 2,3-dioxygenase
i.p.	intraperitoneally
i.v.	intravenously
KAT II	kynurenine aminotransferase II
KP	kynurenine pathway
KYN	kynurenine
KYNA	kynurenic acid
LTP	long-term potentiation
MI	primary motor cortex
MIc	contralateral primary motor cortex
MI_I	ipsilateral primary motor cortex
nACh	nicotinic acetylcholine
NAD	nicotinamide adenine dinucleotide
NMDA	N-methyl-D-aspartate
PND	postnatal day
PROB	probenecid
PTZ	pentylenetetrazole
QUIN	quinolinic acid
TRP	tryptophan

Summary

The kynurenine pathway is the main pathway of the tryptophan metabolism. This metabolic cascade is well known to be responsible for nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate which participate in basic cellular processes.
Some 25 years ago, it was found that intermediates of this pathway have neuroactive properties.
The central compound is the L-kynurenine which in turn can be converted to the excitatory amino acid receptor antagonist kynurenic acid or the N-methyl-D-aspartate receptor agonist quinolinic acid.
Quinolinic acid is a neurotoxic agent which can augment the overexcitation of these receptors and can therefore cause neuronal damage. It has similar neurotoxic effects to those of glutamate in the neocortex, striatum and hippocampus. This glutamate-mediated excitotoxic damage can cause abnormalities in cellular Ca^{2+} homeostasis, elevated production of reactive oxygen species, and induce lipid peroxidation, too.
These processes of cell damage may be prevented by using another metabolite of this pathway, the kynurenic acid.
Kynurenic acid which is one of the few, known endogenous broad-spectrum antagonist of excitatory amino acid receptors, especially the N-methyl-D-aspartate receptors, behaves as a neuroprotective agent: it can inhibit the overexcitation of these receptors by binding the glycine allosteric site. Mor eover, kynurenic acid non-competitively blocks the activity of presynaptic α7-nicotinic acethylcholine receptors.
Changes in the absolute or relative concentration of these compounds i.e. disturbances of the kynurenine pathway in the brain have been implicated in numerous neurodegenerative disorders, such as stroke, epilepsy, ischemia, Alzheimer's and Huntington's diseases, AIDS, multiple sclerosis, encephalopathies, anxiety, depression and schizophrenia.
It is extremely important that the kynurenine pathway abnormality should be suitably prevented or corrected which could attenuate the pathological processes.
Therapeutic use of kynurenic acid is difficult enough since it has a very limited ability to cross the blood-brain barrier. The aim is to develop different analogues of kynurenic acid which can

readily cross the barrier and display similar effectiveness on the affected receptors to that of kynurenic acid.

Another possibility may be the use of its prodrug, L-kynurenine, which can pass the blood-brain barrier easily, and through the action of kynurenine aminotransferase, can increase the brain level of kynurenic acid.

First, we examined the effect of a relatively high dose of L-kynurenine which might lead to an elevated concentration of kynurenic acid in the brain. This effect can be augmented by using probenecid, which prevents the discharge of the organic acids from the cerebrospinal fluid. This proved effective: 300 mg/kg kynurenine administered intraperitoneally slightly decreased the population spike amplitude of the CA1 pyramidal cell responses. Probenecid had a stronger effect with a longer delay: 2 h after the drug injection the amplitude of the evoked potentials marked decreased.

Two compounds administered together resulted in a significant and progressive decrease in amplitude of the population spike evoked on the CA1 pyramidal cells already 30 min after the administration. This effect is probably based on the inhibition of the N-methyl-D-aspartate receptors of the CA1 pyramidal cells by the elevated level of kynurenic acid in the brain tissue, as a consequence of peripheral kynurenine and probenecid administration.

Pentylenetetrazole is often used experimentally to induce seizures which mimic generalized clonic seizures in humans. 60 mg/kg of this drug, administered intraperitoneally to anaesthetized rats, resulted in a significant increase in amplitude of the CA1 spike activity. In some cases, this increase reached the 200% of the control level.

This effect of pentylenetetrazole was completely compensated by pretreatment with kynurenine+probenecid.

In parallel with the electrophysiological results, we studied the behavioral effects of kynurenine administered together with probenecid 2 h before the pentylenetetrazole injection. This convulsive dose of pentylenezetrazole (60 mg/kg) caused epileptic seizures and death in awake animals. It was found that kynurenine and probenecid pretreatment completely protects awake rats from pentylenetetrazole-induced clonic-tonic seizures and death. Although they exhibited reduced rearing, washing and defecation activity, we did not observe any difference in behavior between the controls and the treated animals in the open-field arena. In the water-

maze task, kynurenine+probenecid+pentylenetetrazole-treated animals revealed a significantly impaired performance.

It is well known that kynurenic acid can cross the blood-brain barrier only very poorly; hence, its use as a neuroprotective agent is rather difficult. The Department of Comparative Physiology and the Institute of Medical Chemistry at the University of Szeged have developed a new kynurenic acid analogue, glucoseamine conjugate of kynurenic acid, which probably can readily cross the blood-brain barrier, separate glucosamine and kynurenic acid in the brain, and increase the kynurenic acid level in the central nervous system.

To check the similar effects, we tested these drugs in behavioral and electrophysiological tasks, too.

In agreement with the literature findings, the behavioral experiments revealed that intracerebroventricularly microinjected kynurenic acid induces stereotype behaviour and ataxia. Similar changes in behaviour were found after equimolar administration of glucosamine kynurenic acid.

To examine whether glucosamine kynurenic acid can pass the blood-brain barrier, we administered this drug intravenously and intraperitoneally in the electrophysiological tests. Kynurenic acid administered systemically in doses of 17, 34, 68 or 136 μmol/kg did not cause any observable change in the electrophysiological activity of the hippocampal CA1 region in anaesthetized animals. Against this, glucosamine kynurenic acid in a dose of 136 μmol/kg resulted in the death of the animals in all cases, while a dose of 68 μmol/kg administered intravenously induced the stoppage of breathing of the animals, though they could be resuscitated. A dose of glucosamine kynurenic acid as small as 17 μmol/kg was effective in reducing the CA3 stimulation-evoked activity of the CA1 pyramidal cells in the hippocampus. This effect was augmented when glucosamine kynurenic acid was administered together with probenecid.

N-methyl-D-aspartate receptors play an important role in spatial learning and working memory. The perinatal blockade of these receptors might have behavioral consequences when tests are made at a more advanced age. In the course of our working, we tested the hypothesis whether even a minimal conflict with the N-methyl-D-aspartate receptors in the early critical age of life, which results in mild detectable change in daily behaviour, induces hidden but life-long dysfunctions that can be detected in different parts of the central nervous system. We

chose a low dose of MK-801 (0.1 mg/kg), injecting twice a day on postnatal days 7-19, which caused a slight, not significant impairment of the performance in the water-maze task. Later, these animals were used in electrophysiological examinations.

An increasing number of results suggest that transiently reduced inhibition (e.g. after nerve injury) is a necessary, but not sufficient condition for the development of motor cortex plasticity. The potential for plasticity in the motor cortex has been closely linked to the function of N-methyl-D-aspartate receptors. Simple recordings of evoked potentials in this cortex did not reveal significant differences between the controls and the MK-801-treated animals. Therefore, we tested a more complex phenomenon. The plasticity of evoked responses induced by facial nerve injury can be tested by the paired pulse paradigm. In the control rats, the responses evoked in the motor cortex of both hemispheres by continuous 1-Hz trigeminal stimulation were facilitated after facial nerve transection. This was not the case with the MK-801-treated animals. In a majority of cases, the evoked responses did not change, while in 40% of the cases, continuous stimulation reduced the evoked responses recorded after facial nerve injury.

These results and the related literature indicate the importance of the normal function of the kynurenine pathway and the possibilities of neuroprotection with kynurenine derivatives. Elevated levels of kynurenic acid or its analogues in the brain may reduce the overactivation of excitatory amino acid receptors and may modify or arrest the progression of various neurodegenerative disorders. This can offer a novel therapeutic opportunity where the development of these compounds promises a key for brain neuroprotection.

Introduction

The kynurenine (KYN) pathway (KP) is the main pathway of the tryptophan (TRP) metabolism. All the metabolites of the KP are derived directly or indirectly from KYN, which is the major degradation product of TRP (Fig.1).

This metabolic cascade is well known to be responsible for nicotinamide adenine dinucleotide (NAD) and NAD-phosphate, which participate in basic cellular processes.

Some 25 years ago, it was found that intermediates in the KP have neuroactive properties. It was demonstrated that convulsions appeared in mice after they received quinolinic acid (QUIN) intracerebroventricularly (i.c.v.) (Lapin, 1978). In the same year, the quantitative analysis of L-KYN was investigated by using high-pressure liquid chromatography. It was found that KYN was formed from TRP in the brain (40%) and was also taken up from the periphery (60%). The rate of cerebral KYN synthesis was 0.29 nmol/g/h (Gal and Sherman, 1978). Moreover, it is well known that KYN can easily cross the blood-brain barrier (BBB) with the aid of neutral amino acid carriers (Fukui *et al.*, 1991).

Lapin was one of the pioneers of studies of the KYN pathway and its metabolites. In 1973, he presumed that KYNs are probable participants in depression (Lapin, 1973). Furthermore, L-KYN plays an important role in the modulation of female sexual behaviour: lordosis was facilitated in oestrogen-primed ovariectomized rats by the administration of L-KYN into the ventricles (Mendelson *et al.*, 1987).

The L-KYN derivative QUIN (Fig.1) is a selective ligand of the N-methyl-D-aspartate (NMDA) receptor (Stone and Perkins, 1981), which can therefore cause neuronal damage (Schwarcz *et al.*, 1984). It has similar neurotoxic effects to those of glutamate in the neocortex, striatum and hippocampus (Perkins and Stone, 1983).

It was alre ady hypothesized in 1983 that QUIN plays an important role in Huntington's disease (Schwarcz *et al.*, 1983). Three years later, Beal et al. proved that the injection of QUIN into the rat striatum duplicated the neurochemical features of this disorder (Beal *et al.*, 1986).

The convulsant and neurodegenerative properties of QUIN are especially pronounced in the hippocampus (Schwarcz et al., 1987; Tsuzuki et al., 1989b). The QUIN-induced depolarization and burst firing of the CA1 pyramidal neurons are Ca^{2+}-dependent (Peet *et al.*,

1986) and QUIN can cause a marked increase in the intracellular Ca^{2+} concentration even after the voltage-dependent Ca^{2+} channels have been suppressed (Tsuzuki et al., 1989a).

Intrastriatal injection of QUIN induces a substantial neuronal loss, which is potentiated by the administration of 3-hydroxykynurenine (3-HK) (Guidetti and Schwarcz, 1999). It produces early changes in the activity of the striatal neurons and movements of several cations, which may contribute to subsequent abnormalities in energy metabolism and to cell death (Bordelon *et al.*, 1998).

Bilateral intrastriatal microinjection of QUIN induced hyperactivity and weight loss (Sanberg *et al.*, 1989), increased nocturnal locomotor activity (Bazzett *et al.*, 1996) and abnormal feeding behaviour (Giordano *et al.*, 1990). Furthermore, QUIN-lesioned animlas displayed an impaired acquisition in the visuospatial skills, in the transfer of learning and in the retrieval of stored memories in the Morris water-maze test (Furtado and Mazurek, 1996; Joel *et al.*, 1998; Francis *et al.*, 2000), and in the spatial learning in the radial-maze test (Shear *et al.*, 1998).

The other important metabolite of KP is kynurenic acid (KYNA) (Fig.1), which is one of the few, known endogenous broad-spectrum antagonists of excitatory amino acid (EAA) receptors (Swartz *et al.*, 1990), especially the NMDA receptors. KYNA behaves as a neuroprotective agent: it can inhibit the overexcitation of these receptors by binding the glycine allosteric site. It may therefore influence physiological and pathological processes, so it has therapeutic effects in numerous neurological disorders (Stone, 2000; Stone, 2001; Stone *et al.*, 2003). Furthermore, KYNA displays neuroinhibitory properties in neurophysiological experiments (Perkins and Stone, 1982) and, like other glutamate receptor antagonists, can cause impairments in learning and behaviour (Morris *et al.*, 1986).

An early study revealed that the 2-carboxy and 4-hydroxy groups of KYNA (Fig. 1) are essential for the antagonist activity (Robinson *et al.*, 1985).

In 1988, Moroni et al. reported that the concentration of KYNA in the mammalian brain is in the range 10-150 nmol (Moroni *et al.*, 1988), and some years later pharmacological studies proved that KYNA is derived in most brain regions primarily from kynurenine aminotransferase II (KAT II) activity (Guidetti *et al.*, 1997), which is located mainly in the glia (Du *et al.*, 1992). The glia has uptake mechanisms for KYN and the ability to release KYNA (Turski *et al.*, 1989; Speciale and Schwarcz, 1990). KAT II operates best at physiological pH and preferentially recognizes KYN as substrate (Okuno *et al.*, 1991). L-

Cysteine sulphinate, an endogenous sulphur-containing amino acid, inhibits the activity of KAT II, decreases the KYNA production in the brain and has a higher potency than that of any other known KAT II inhibitor (Kocki *et al.*, 2003).

Moreover, KYNA non-competitively blocks the activity of presynaptic α7-nicotinic acetylcholine (nACh) receptors and can increase the expression of non-α7-nACh receptors (Hilmas *et al.*, 2001). Cross-talk between KYNA and the nicotinic cholinergic system has been presumed to play a role in the pathogenesis of numerous brain disorders, including schizophrenia and Alzheimer's disease, in which brain KYNA levels are elevated and nicotinic functions are impaired (Alkondon *et al.*, 2004).

KYNA can suppress spontaneous epileptiform burst discharges (Stone, 1988) and depress the amplitude and duration of Mg^{2+}-free bursts (Schneiderman and MacDonald, 1989) in the CA3 region of hippocampal slices.

Long-term potentiation (LTP), which is associated with the NMDA receptor function, is dramatically enhanced by glycine in area CA1 in slices and this effect can be antagonized by KYNA (Tauck and Ashbeck, 1990). Additionally, NMDA receptor blockers reversibly blocked the induction of LTP in the commissural/associational pathway, but had no effect on mossy fibre LTP (Harris and Cotman, 1986).

Intraraphe administration of KYNA resulted in marked hyperactivity (Wirtshafter *et al.*, 1989), while its bilateral administration into the nucleus accumbens increased the number of visits to unbaited arms (reference memory errors) (Schacter *et al.*, 1989), impaired the visual discrimination and enhanced the motor activity (Ericson *et al.*, 1990).

3-HK and anthranilic acid (ANA) (Fig. 1) cause neuronal damage by producing oxygen free radicals and raising the levels of oxidative stress and lipid peroxidation. These can contribute to the impairment of the mitochondrial function (Lee *et al.*, 2004), including the heart mitochondria, which can lead to cardiovascular abnormalities (Baran *et al.*, 2003). Moreover, 3-HK may potentiate the neurotoxic effects of QUIN (Guidetti and Schwarcz, 1999) resulting in neuronal cell death. This may be attenuated by epigallocatechin 3-gallate, a major compound in green tea (Jeong *et al.*, 2004).

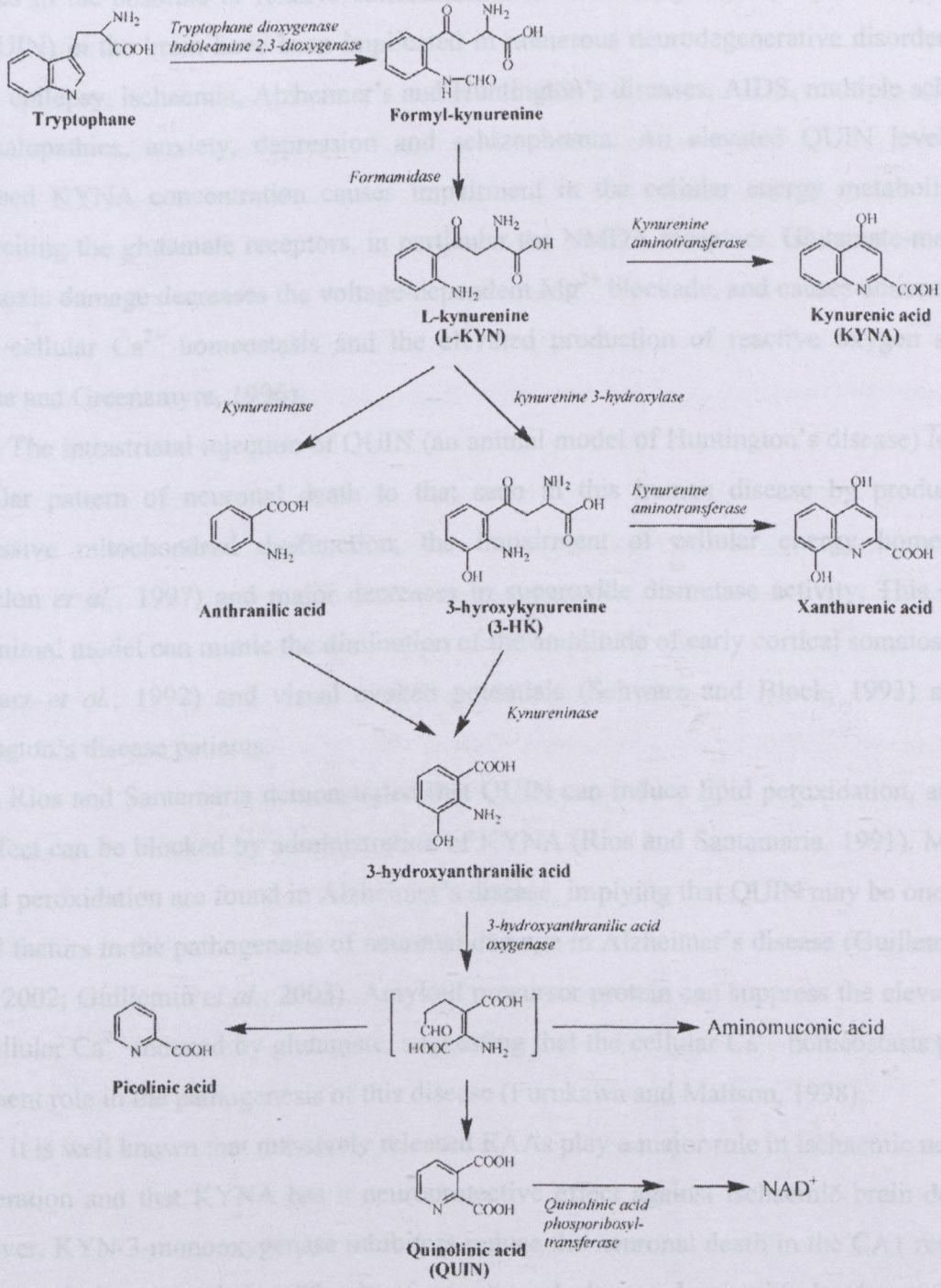

Figure 1. The kynurenine pathway

Changes in the absolute or relative concentration of these compounds (in particular KYNA and QUIN) in the brain have been implicated in numerous neurodegenerative disorders, e.g. stroke, epilepsy, ischaemia, Alzheimer's and Huntington's diseases, AIDS, multiple sclerosis, encephalopathies, anxiety, depression and schizophrenia. An elevated QUIN level or a decreased KYNA concentration causes impairment in the cellular energy metabolism by overexciting the glutamate receptors, in particular the NMDA receptors. Glutamate-mediated excitotoxic damage decreases the voltage-dependent Mg^{2+} blockade, and causes abnormalities in the cellular Ca^{2+} homeostasis and the elevated production of reactive oxygen species (Greene and Greenamyre, 1996).

The intrastriatal injection of QUIN (an animal model of Huntington's disease) leads to a similar pattern of neuronal death to that seen in this human disease by producing a progressive mitochondrial dysfunction, the impairment of cellular energy homeostasis (Bordelon *et al.*, 1997) and major decreases in superoxide dismutase activity. This widely used animal model can mimic the diminution of the amplitude of early cortical somatosensory (Schwarz *et al.*, 1992) and visual evoked potentials (Schwarz and Block, 1993) seen in Huntington's disease patients.

Rios and Santamaria demonstrated that QUIN can induce lipid peroxidation, and that this effect can be blocked by administration of KYNA (Rios and Santamaria, 1991). Markers of lipid peroxidation are found in Alzheimer's disease, implying that QUIN may be one of the critical factors in the pathogenesis of neuronal damage in Alzheimer's disease (Guillemin and Brew, 2002; Guillemin *et al.*, 2003). Amyloid precursor protein can suppress the elevation of intracellular Ca^{2+} induced by glutamate, suggesting that the cellular Ca^{2+} homeostasis plays a prominent role in the pathogenesis of this disease (Furukawa and Mattson, 1998).

It is well known that massively released EAAs play a major role in ischaemic neuronal degeneration and that KYNA has a neuroprotective effect against ischaemic brain damage. Moreover, KYN-3-monooxygenase inhibitors reduce the neuronal death in the CA1 region of hippocampal slices exposed to 30 min of oxygen and glucose deprivation by decreasing the local synthesis of 3-HK and QUIN (Carpenedo *et al.*, 2002). Administration of KYN produces a significant increase in the normal and ischaemic corticocerebral blood flow, which can be prevented by pretreatment with atropine or N-omega-nitro-L-arginine, suggesting that this

process might be mediated by the activation of cholinergic and nitrogen monoxide pathways (Sas *et al.*, 2003).

Even low concentrations of endogenous KYNA can reduce the number of hippocampal slices with spontaneous epileptiform discharge after exposure to a buffer lacking Mg^{2+}. It is suggested that endogenous KYNA plays an important role in the suppression of seizure-like activity (Scharfman *et al.*, 2000). Furthermore, in a genetic model of absence epilepsy, a significantly lower concentration of KYNA was found in the frontal cortex than in the non-epileptic control (Kaminski *et al.*, 2003). A dysfunction of the serotoninergic system, including the KP, has been found in patients with temporal lobe epilepsy (Natsume *et al.*, 2003) or with the tuberous sclerosis complex (Fedi *et al.*, 2003).

An abnormality of the KP has also been found in neuropsychiatry: a disturbed metabolism of TRP affects the biosynthesis of the neurotransmitter 5-hydroxytriptamine and this appears to be associated with an increased susceptibility to depression. The activation of indolamine 2,3-dioxygenase (IDO) (Fig. 1) could be an important link between the immunological network and the pathogenesis of depression (Widner *et al.*, 2002), while reduced TRP availability plays a role in interferon-alpha-induced depressive symptoms (Capuron *et al.*, 2003). In schizophrenic patients an elevated level of endogenous KYNA may be demonstrated (Erhardt and Engberg, 2002).

It is extremely important that the KP abnormality should be suitably prevented or corrected which could attenuate the pathological processes (mentioned above).
There are at least two ways in which therapeutic agents are being developed with the aim of modulation of the KP.

One approach is to use analogues of KYNA as antagonists at glutamate receptors, because KYNA is able to pass the BBB only poorly, which hampers its use. The aim here is to develop different analogues of KYNA which can readily cross the BBB and display similar effectiveness on the affected receptors to that of KYNA.

The second approach to fend off the effects of a KP disturbance is to use enzyme inhibitors which can decrease the activities of the enzymes that facilitate the QUIN formation. These enzyme inhibitors can therefore shift the KP towards the neuroprotective KYNA and inhibit the accumulation of QUIN and other neurotoxic metabolites.

Some synthetic KYNA derivatives can behave as antagonists of NMDA receptors and provide an attractive strategy for the development of novel neuroprotective and anticonvulsive agents. 7-chloro-KYNA (7-Cl-KYNA) is a potent selective antagonist at the glycine site (Kemp *et al.*, 1988), but its penetration through the BBB is poor. Its prodrug, 4-chloro-KYN (Wu et al., 1997a), readily enters the brain from the circulation and prevents QUIN-induced neurotoxicity in the rat hippocampus (Wu *et al.*, 2000) and striatum (Lee and Schwarcz, 2001) after systemic administration. 7-Cl-KYNA depresses or completely blocks the NMDA receptor-mediated synaptic transmission in a Mg^{2+}-free medium and the induction of LTP in a Mg^{2+}-containing medium in the CA1 region of rat hippocampal slices (Bashir *et al.*, 1990; Izumi *et al.*, 1990; Thiels *et al.*, 1992), and it also inhibits the induction of LTP following tetanic stimulation (Watanabe *et al.*, 1992; Ikegaya *et al.*, 1995). A similar effect was observed in the neonatal rat hippocampus, related with theta burst stimulation (Oliver *et al.*, 1990).
D-glucose or D-galactose esters of 7-chloro-KYNA have been synthetized to facilitate the transport of 7-Cl-KYNA across the BBB (Battaglia *et al.*, 2000).

The following aims were set during our work on this topic:

To establish

- whether L-KYN, which can cross easily the BBB and can be transformed into neuroprotective KYNA in the brain, can act against the epileptic seizures induced by pentylenetetrazole (PTZ);

- whether L-KYN administered intraperitoneally (i.p.) together with probenecid (PROB), a known inhibitor of the transport of organic acid from the cerebrospinal fluid (CSF), can be more effective than KYN administered alone and whether they are able to protect against the neurotoxic effect of PTZ;

- whether the newly synthetized glucosamine-kynurenic acid (G-KYNA) administerd i.c.v. has similar neuroprotective effects on the NMDA receptor complex to those of KYNA;

- whether systemically administered G-KYNA is able to cross the BBB and can be more effective than KYNA injected i.p.;

- whether neonatal treatment with MK-801, a non-competitive NMDA receptor antagonist, induces any changes in spatial learning memory and in the evoked potentials of the motor cortex in adult rats.

Material and Methods

1. Animals

Male Wistar rats (220-280g) were housed individually and had free access to food and water. All efforts were made to minimize animal suffering. The principles of laboratory animal care (NIH publication No. 85-23) and the protocol for animal care approved by the European Communities Council Directive of 24 November 1986 (86/609/EEC) were followed.

2. Drugs

KYN, KYNA, PROB, PTZ and MK-801 were obtained from Sigma (Steinheim, Germany), while the new compound G-KYNA (Fig. 2) was synthetized in the Institute of Medical Chemistry, University of Szeged.

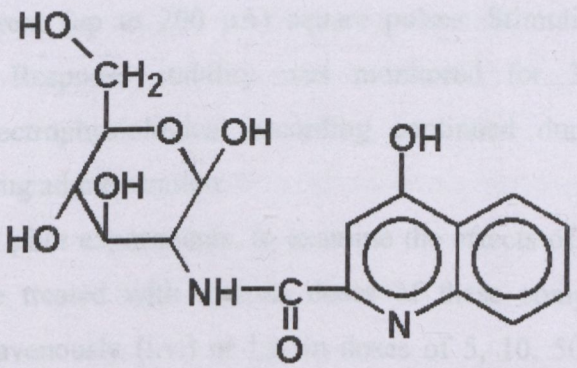

Figure 2. The chemical structure of G-KYNA

3. Electrophysiology

3.1. Hippocampal CA3 area stimulation and contralateral CA1 area registration

Surgical procedure: Animals were anaesthetized with urethane (1.25 g/kg, i.p.). In some cases, the tail vein of the animals was catheterized; in most of the experiments, however, the drugs were administered i.p. through a syringe implanted at the beginning of the experiments. For recordings in area CA1, a 2-3 mm-diameter hole was drilled over the dorsal hippocampus (3.0-3.8 mm posterior to the bregma and 1.8-2.3 mm lateral to the sagittal suture) and the recording electrode was lowered 2.4-3.6 mm from the cortical surface. Contralaterally, a 1-2-mm hole was drilled for the CA3 stimulating electrode (3.7 mm posterior to the bregma, and 3.3 mm lateral to the sagittal suture; final electrode depth 3.8 mm below the dura). Electrodes were lowered and final positions were adjusted so that the maximum CA1 population spike was obtained in response to contralateral CA3 stimulation (Fig. 3). The sites in areas CA1 and CA3 were confirmed histologically. Responses to a range of stimulus intensities were recorded under control conditions to produce an input-output curve by changing the duration (10-100 μs), using current (up to 200 μA) square pulses. Stimuli were triggered at low frequency (0.05 Hz). Response stability was monitored for 30 min prior to drug administration. The electrophysiological recording continued during the following 3-h recording period after drug administration.

Drug administration: In pilot experiments, to examine the effects of pure KYN or PROB or PTZ, the animals were treated with various doses of these compounds, e.g. KYN was administered either intravenously (i.v.) or i.p. in doses of 5, 10, 50, 100, 200, 300 or 400 mg/kg. Since i.p. and i.v. administration resulted in similar effects, i.p. administration was chosen in further studies, because it was more appropriate in the behavioral experiments. On the basis of the literature data (Vecsei et al., 1992; Wu et al., 1997b) and our pilot experiments on the dose-dependent effects of these compounds, we chose KYN in a dose of 300 mg/kg, PROB in a dose of 200 mg/kg and PTZ in a dose of 60 mg/kg throughout the main study.

The chosen doses of KYNA and G-KYNA were also based on earlier pilot experiments, in which both KYNA and G-KYNA were administered i.v. or i.p., in doses of 17, 34, 68 or 136 μmol/kg. Since i.p. and i.v. administration resulted in similar effects, i.p. administration was

chosen in further studies, because this seemed to be more appropriate in the scheduled behavioral experiments. We endeavoured to find the minimum doses that were still effective: accordingly, on the basis of these pilot experiments, both KYNA and G-KYNA were administered in a dose of 17 μmol/kg throughout the main study.

3.2. Cortical evoked potentials

Surgical procedure: Rats were anaesthetized with a mixture of Ketavet (10.0 mg/100 g) and Rompun (xylazine, 0.8 mg/100 g). On both sides, the primary motor cortex (MI) was exposed by craniotomy from about 2 mm posterior to 5 mm anterior from the bregma, and from 0.5 to 5 mm lateral from the midline. In the course of the operation, we also exposed the right side facial nerve, including its postauricular branch. This was later transected, during the electrophysiological recordings.

Electrical stimulation of the vibrissa pad or electromechanical vibrissa stimulation was employed to induce evoked potentials in the MIs in both hemispheres: the right whisker pad was stimulated electrically with bipolar needle electrodes (1 Hz; 0.3-ms duration, 150-200 μA) to evoke visible whisker movements, whilst for mechanical stimulation whiskers were deflected by using a multiangle electromechanical stimulator. To investigate paired pulse inhibition effects on the amplitudes of evoked potentials, a paired-pulse stimulation protocol was used (application of two electrical pulses with a 200-ms interstimulus interval). The ratio of the amplitudes of the evoked potentials elicited by the second versus the first stimulus was calculated and defined as the Q value (e.g. Q=EP2/EP1). Q<1 means that the second response was inhibited by the first one.

4. Behaviour

4.1. Open-field observations

This behavioural test was carried out in a circular arena (80 cm in diameter); the height of the wall was 40 cm. Following administration of the compounds, the animals were observed for 5 min. The stereotyped behaviour was characterized by the total time spent in grooming and

washing. Further parameters were monitored in different experiments: time spent in centre and periphery, rearing, defecation, the time (s) to the onset of grand mal seizures, the time (min) of death or ataxia. For ataxia, the ratings were '1': awkward and jerky movements; '2': stumbling or an awkward posture; '3': falling, '4': cannot move beyond a small area or supports weight on stomach or haunches; and '5': unable to move except for twitching movements.

4.2. Water-maze task

The rats were trained in a large circular swimming pool (160 cm in diameter, 60 cm in height) filled with water to a depth of 35 cm. The water was at room temperature and was made opaque by the addition of 2 l of milk. The pool was situated in a small rectangular room. The walls were equipped with a variety of spatial cues (pictures and a lamp emitting diffuse light), which remained unchanged during the experiment. The pool was divided into four quadrants, and a removable platform (8 cm in diameter) was hidden at one of four positions in the pool, exactly 25 cm from the side wall. The platform was 1.0 cm below the water surface and not visible for the swimming rat. The animal performed a block of four consecutive trials, all beginning at a fixed starting point (N, W, S or E). Trials ended either when the platform was found or when 60 s had elapsed. If the rats did not find the platform within 60 s, they were guided to it and left there for 15 s. They were then removed from the pool and either placed back after 15 s (intertrial interval) for the subsequent trial or returned to their home cages after being dried with a towel. The 5-day training consisted of one block of four trials per day, and for each trial the starting point varied in a random order such that no location recurred for a given day. Each trial was recorded and analysed by using a computer video tracking system. The video camera of the *Ethovision System* (Noldus) was mounted above the centre of the pool.

To check the condition of the sensory and motor system of the animals, we can use the visual cue task. In these experiments, the platform is above the surface of the water so that it is visible for the swimming animals. If there is no significant difference in the performances of the treated and control animals in the visual cue task, we may conclude that the sensory and motor systems are in working order and any difference in performance is due to the impaired memory function.

5. Data analysis

For hippocampal evoked potentials, statistical significance was determined by means of the *Student t*-test (p value set at 0.05 for significance).

For the MI evoked potentials and the behavioral experiments, the statistical analysis was performed by using repeated measures ANOVA (SPSS for Windows 9.0).

Results

1. Electrophysiology

1.1. Hippocampal CA3 area stimulation and contralateral CA1 area registration

In these experiments, we decided to focus on the hippocampus: the evoked responses of the hippocampal neurones were chosen as end-point of these electrophysiological experiments because of the high concentrations of glutamate receptors on the dendrites of these neurones (Martin *et al.*, 1993; Watanabe *et al.*, 1993; Monyer *et al.*, 1994) and because they receive glutamatergic afferents that can be stimulated preferentially *in vivo* (Fig. 3). In addition to electrophysiological experiments, the behavioral effects of these compounds (KYN, PROB and PTZ) were also studied in an open-field arena and in a water-maze task, which is likewise a suitable test for the hippocampal function.

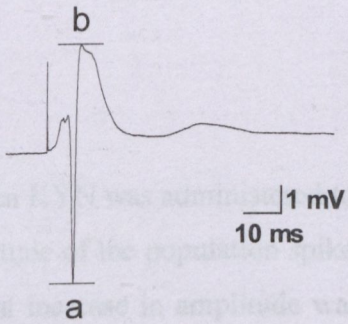

Figure 3. Population spike recorded in the CA1 area of the rat hippocampus. a: minimum point, b: maximum point of the response. b-a = amplitude of the population spike.

1.1.1. Effects of KYN

The responses of the area CA1 pyramidal cells to contralateral CA3 stimulation were tested before and after the injection of KYN. KYN induced some changes in the population spike amplitudes in all cases. This occurred regardless of the mode of application, e.g. both i.v. and i.p. injections. However, the effects of KYN were controversial: in some experiments, the

systemically administered KYN resulted in a transient facilitation of the population spike amplitudes, while in other cases the same dose of this compound, administered in the same way, transiently decreased the amplitudes of the population spikes. These changes in amplitude rarely reached the level of significance (Fig. 4A). To summarize the overall data on KYN (300 mg/kg), it may be stated that KYN did not significantly change the amplitude of the evoked responses (Table 1).

1.1.2. Effects of PROB

PROB is a known inhibitor of the transport of organic acid from the CSF (Cunningham *et al.*, 1981; Vecsei *et al.*, 1998) and hence can increase the KYNA concentration in the brain. When administered in a dose of 200 mg/kg, it did not cause an immediate significant change in the amplitude of the evoked population spike activity. In most cases, it resulted in a decrease in amplitude, but only 2 h after the administration (Fig. 4B). The overall data for all the animals in this group indicate a moderate inhibitory effect of PROB with a long delay (Table 1).

1.1.3. Effects of KYN+PROB

The results were quite different when KYN was administered together with PROB. In all these cases, a significant change in amplitude of the population spikes occurred within 5-10 min. In some cases, a short, slight, transient increase in amplitude was observed after application of the drugs, but this was followed in all cases by a significant decrease in amplitude (Fig. 4C). In most cases, only the characteristic decrease in amplitude could be detected, as shown by the overall data for the animals in this group (Table 1).

1.1.4. Effects of PTZ

PTZ in a dose of 60 mg/kg induced a significant increase in amplitude of the population spikes registered in the CA1 area. This increase in amplitude started just after the drug administration, reached its plateau within 10 min and remained at the elevated level during the 3-h registration period (Fig. 4D and Table 1). In one of the five PTZ-treated animals, the

amplitude increased to over 200% of the control level (Fig. 4D); in the other four animals, the increment changed by 115-150% of the control amplitude. The administration of PTZ (60 mg/kg) never resulted in the death of the anaesthetized animals.

1.1.5. Effects of KYN+PROB+PTZ

It was stated above that PTZ (60 mg/kg) induced an immediate and significant increase in amplitude of the CA1 responses in all of the experiments. This PTZ-induced enhancement in amplitude was completely blocked in those animals in which KYN+PROB administration preceded the injection of PTZ (Fig. 4E). KYN (300 mg/kg) + PROB (200 mg/kg) were injected 2 h before the PTZ injection. The data obtained from the five PTZ-treated animals that received KYN+PROB prior to PTZ administration clearly demonstrate that KYN+PROB did not allow the development of the amplitude increase following PTZ administration (Table 1).

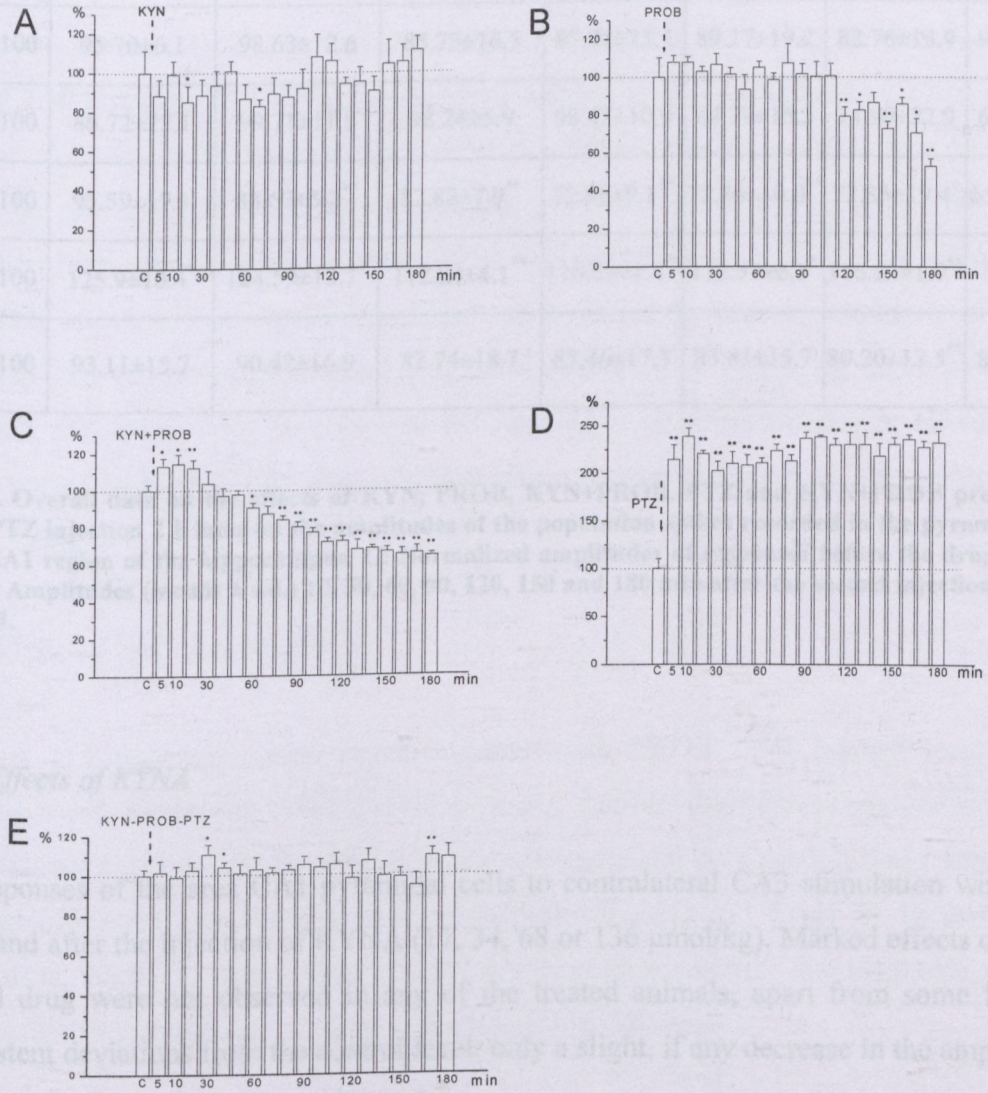

Figure 4. Examples of the effects of the administered compounds on the population spike amplitudes recorded in the CA1 area. A: After 300 mg/kg KYN injection, hardly any marked change in amplitude was observed. B: 200 mg/kg PROB resulted in a decreased CA1 population spike with a 2-h delay. C: KYN+PROB injection resulted in a rapid and significant decrease in amplitude. D: 60 mg/kg PTZ resulted in a 200% increase in amplitude of the population spike. E: Effect of KYN+PROB+PTZ on the population spike recorded in the CA1 area. Ordinate: Spike amplitudes as percentages of the control. Abscissa: Time in min after drug injection. Each column represents the mean ± s.d. for 5 potentials. *p<0.05, **p<0.01.

	C / min	10	30	60	90	120	150	180
KYN	100	95.70±6.1	98.63±12.6	85.73±16.5	87.91±15.5	89.17±19.2	82.76±13.9	90.74±19.6
PROB	100	86.72±22.1	99.71±11.8	98.24±5.9	98.43±10.9	84.79±16.5	74.90±22.9	68.56±23.2
KYN+ PROB	100	95.59±17.1	88.67±5.2**	82.82±7.9**	72.98±9.1**	72.26±10.0**	72.85±19.4*	65.22±15.9**
PTZ	100	125.9±10.4*	124.59±13.7*	112.23±4.1**	116.69±1.4**	112.55±6.3*	116.23±1.9**	116.5±7.8*
KYN+ PROB +PTZ	100	93.11±15.7	90.42±16.9	82.74±18.7	87.46±17.3	83.81±15.7	80.20±12.5**	88.43±11.9

Table 1. Overall data on the effects of KYN, PROB, KYN+PROB, PTZ and KYN+PROB pretreatment with a PTZ injection 2 h later on the amplitudes of the population spikes recorded in the pyramidal layer of the CA1 region of the hippocampus. C: Normalized amplitudes of responses before the drug injection (100%). Amplitudes (means ± s.d.) 10, 30, 60, 90, 120, 150 and 180 min after the second injection. *p<0.05, **p<0.01.

1.1.6. Effects of KYNA

The responses of the area CA1 pyramidal cells to contralateral CA3 stimulation were tested before and after the injection of KYNA (17, 34, 68 or 136 μmol/kg). Marked effects of the i.p. injected drug were not observed in any of the treated animals, apart from some transient, inconsistent deviations from the control level: only a slight, if any decrease in the amplitude of the population spike (Fig. 5A and Table 2).

1.1.7. Effects of KYNA+PROB

KYNA (17 μmol/kg) + PROB (200 mg/kg) administration together resulted in similar effects to those seen following PROB (200 mg/kg) administration alone. In most cases, when KYNA + PROB were administered together, there was no change in the amplitudes of the responses during 1 h following the drug administration, but, with a 1.5-2-h delay, a slight decrease in amplitude was registered (Fig. 5B and Table 2).

1.1.8. Effects of G-KYNA

In pilot experiments, G-KYNA was administered in doses of 17, 34, 68 or 136 μmol/kg. In a dose of 136 μmol/kg, G-KYNA administered either i.v. or i.p. resulted in 100% mortality within 5 min. Similarly, 68 μmol/kg G-KYNA administered i.v. resulted in a stoppage of breath, but the animals could be resuscitated. I.p. injection of this dose did not stop the breathing of the animals. These findings led us to reduce the dose of the drug to a level as low as possible which still resulted in a marked and clear-cut decrease in amplitude of the CA1 pyramidal cell responses. We found that, in a dose of $\geq$ 17 μmol/kg, G-KYNA induced consistent and appreciable decreases in the population spike amplitudes in all cases. This change started 50-60 min after drug administration, regardless of the mode of application (Fig. 5C and Table 2).

1.1.9. Effects of G-KYNA+PROB

G-KYNA (17 μmol/kg) administered together with PROB (200 mg/kg) resulted in a progressive and, by the end of the recording period, considerable decrease in amplitude of the CA1 population spikes. The decrease in amplitude began and became significant within 10-20 min, and continued over the 3-h registration time (Fig. 5D and Table 2). In some of the experiments, the registration continued over 4.5 h. In these animals, the amplitude reduction was progressive throughout the whole experiment (Fig. 5E).

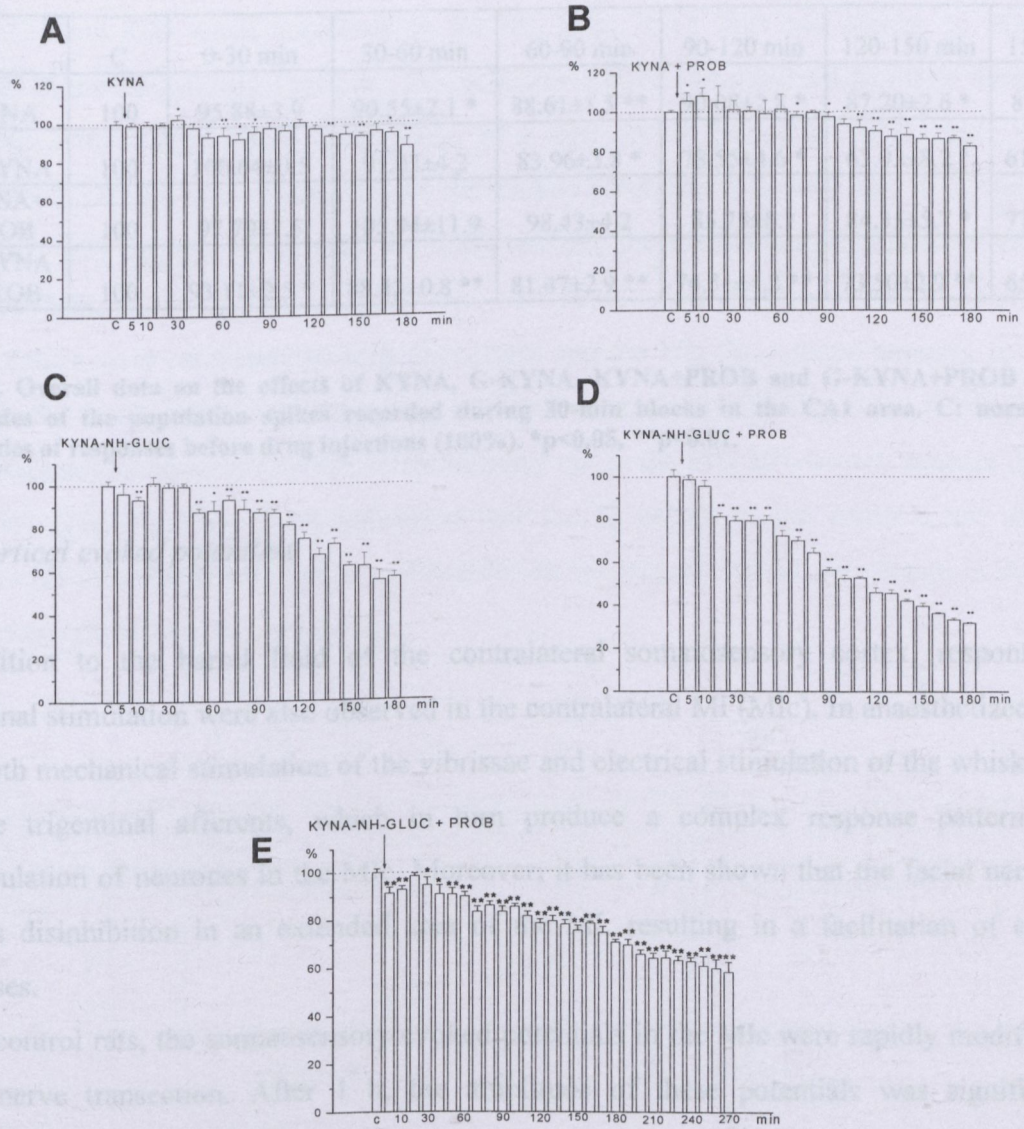

Figure 5. Examples of the effects of the administered compounds on the population spike amplitudes recorded in CA1. A: After KYNA injection, there were some decreases in amplitude, but these changes were not marked. B: After a transient increase, KYNA+PROB resulted in a decreased response with a 1.5-2-h delay. C: G-KYNA injection resulted in marked and significant decreases in the CA1 population spike amplitudes with a 50-60-min delay. D: G-KYNA+PROB i.p. injection resulted in a considerable decrease in amplitude, shortly (10-20 min) after administration. This continuous decrease was maintained during the 4.5-h registration period (E). Ordinate: Spike amplitudes as percentages of the controls. Abscissa: Time in min after drug injection. Each column indicates the mean ± s.d. for 5 potentials. *p<0.05, **p<0.01.

	C	0-30 min	30-60 min	60-90 min	90-120 min	120-150 min	150-180 min
KYNA	100	95.88±3.9	90.55±2.1 *	88.61±1.5 **	90.08±2.8 *	87.20±2.6 *	87.39±2.4 *
G-KYNA	100	100.64±0.5	91.03±4.2	83.96±3.8 *	78.55±4.6 *	63.93±8.2 *	61.65±3.0 **
KYNA+ PROB	100	97.79±1.5	105.94±11.9	98.43±4.2	85.75±8.1	84.35±5.7 *	77.76±1.4 **
G-KYNA +PROB	100	93.11±2.5 *	88.43±0.8 **	81.47±2.9 **	74.51±4.2 **	73.50±2.9 **	65.13±6.6 **

Table 2. Overall data on the effects of KYNA, G-KYNA, KYNA+PROB and G-KYNA+PROB on the amplitudes of the population spikes recorded during 30-min blocks in the CA1 area. C: normalized amplitudes of responses before drug injections (100%). *p<0.05, **p<0.01.

1.2. Cortical evoked potentials

In addition to the barrel field of the contralateral somatosensory cortex, responses to trigeminal stimulation were also observed in the contralateral MI (MIc). In anaesthetized adult rats, both mechanical stimulation of the vibrissae and electrical stimulation of the whisker pad activate trigeminal afferents, which in turn produce a complex response pattern in a subpopulation of neurones in the MIc. Moreover, it has been shown that the facial nerve cut induces disinhibition in an extended area of the MI, resulting in a facilitation of evoked responses.

In the control rats, the somatosensory evoked potentials in the MIc were rapidly modified by facial nerve transection. After 1 h, the amplitude of these potentials was significantly enhanced and the latencies of all the components had shortened. Responses with enhanced amplitudes could be observed throughout the 3- to 4-h recording session (Fig. 6A). Similar effects were found in the ipsilateral MI (MIi): in the control rats, stimulation of the trigeminal nerve or parts of it evoked very small potentials in the MIi; however, a few minutes after the facial nerve injury, evoked potentials could also be elicited with enhanced amplitude in this area, too. Their amplitude increased considerably within 1 h and remained high until the end of the experiments (3-4 h after denervation) (Fig. 6B).

This was not the case in the rats treated with MK-801 as young animals. These animals received injections of 0.1 mg/kg MK-801 (dizocilpine) twice a day, starting on postnatal day (PND) 7 and lasting until PND 19.

In all of these rats, trigeminal stimulation evoked potentials with high amplitude in the MI_C (Fig. 7A1) and with small amplitude in the MI_I (Fig. 7B1). This was similar to what was observed in the control rats. However, the facial nerve transection did not facilitate the evoked responses in the MI in either hemisphere. In a majority of the cases (60%), there was no change in the amplitudes or latencies of the evoked potentials following the facial nerve cut (Fig. 7A2, B2). In fact, in 40% of the cases studied, the amplitudes decreased or vanished from both MIs.

The Q values were calculated in both the control and the MK-801-treated animals. In all the controls, the studies with a paired-pulse paradigm revealed disinhibition ($Q > 1$), while there was hardly any increase in Q after the facial nerve cut in the MK-801-treated animals ($Q<1$ in all cases) (Fig. 8). This is an indication that the Q values in the controls are changed significantly after facial nerve injury (disinhibition occurs), but Q did not change in the MK-801-treated animals (no disinhibition).

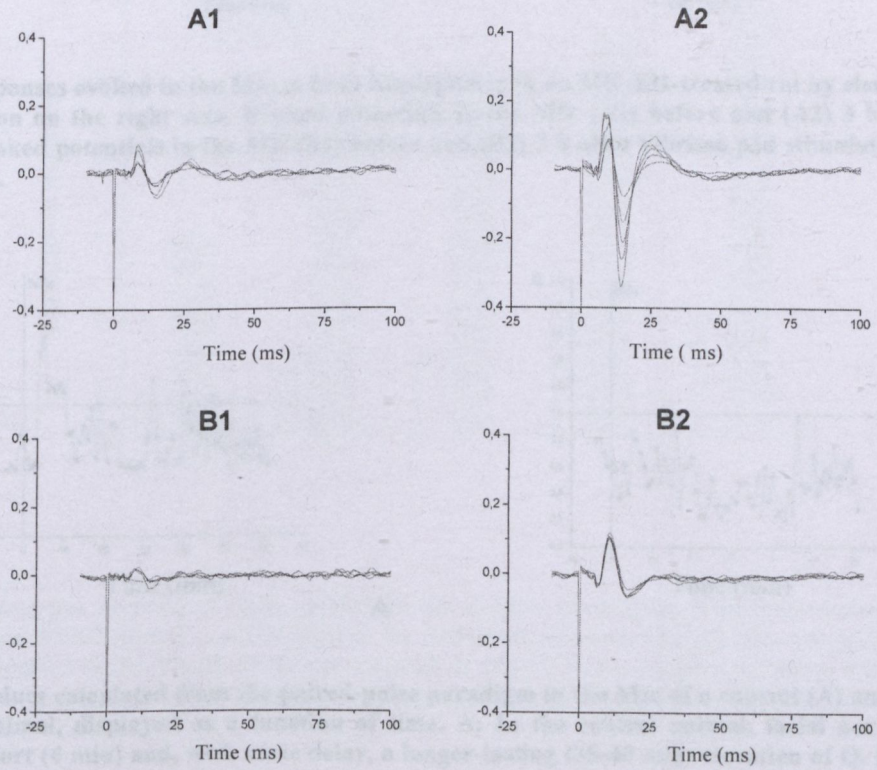

Figure 6. Responses evoked in the MIs in both hemispheres of a control rat by right side electrical vibrissa pad stimulation. Evoked potentials in the MIc recorded (A1) before and (A2) 3 h after a facial nerve cut. Evoked potentials in the MIi (B1) before and (B2) 3 h after vibrissa pad stimulation. Ordinates are in mV/div.

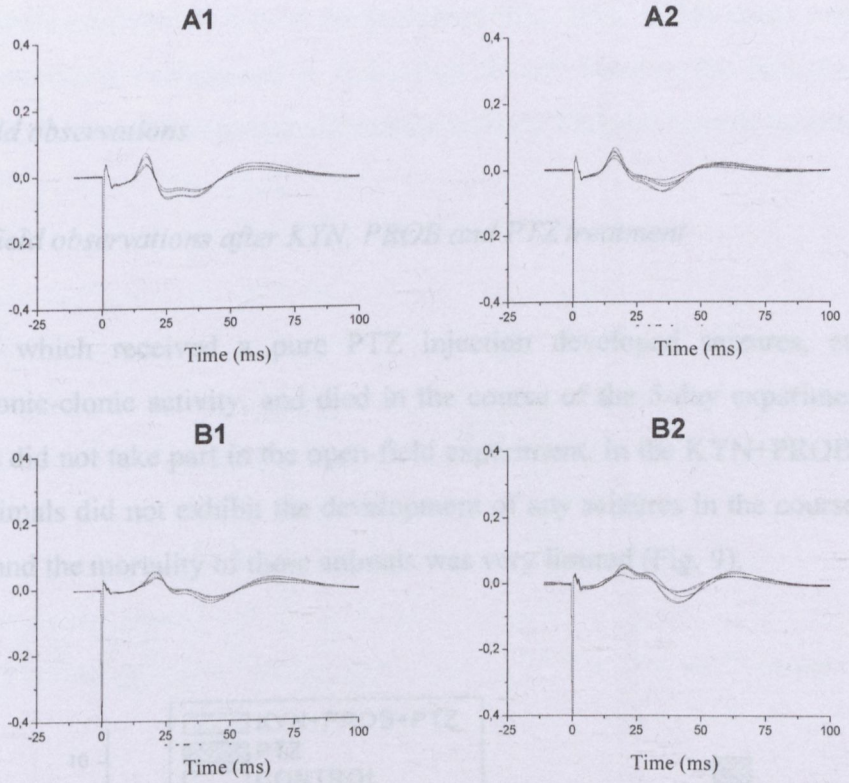

Figure 7. Responses evoked in the MIs in both hemispheres in an MK-801-treated rat by electrical vibrissa pad stimulation on the right side. Evoked potentials in the MIc (A1) before and (A2) 3 h after a facial nerve cut. Evoked potentials in the MIi (B1) before and (B2) 3 h after vibrissa pad stimulation. Ordinates are in mV/div.

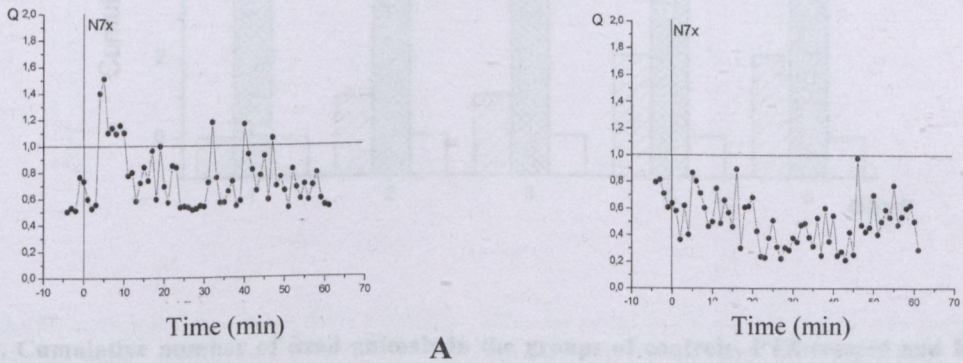

Figure 8. Q values calculated from the paired-pulse paradigm in the MIc of a control (A) and an MK-801-treated (B) animal, displayed as a function of time. A: In the control animal, facial nerve transection produced a short (4 min) and, with some delay, a longer-lasting (35-40 min) elevation of Q. B: In the MK-801-treated animal, transection of the facial nerve did not cause cortical disinhibition: the Q value remained <1. N7x: facial nerve transection

2. Behaviour

2.1. Open-field observations

2.1.1. Open-field observations after KYN, PROB and PTZ treatment

The animals which received a pure PTZ injection developed seizures, culminating in generalized tonic-clonic activity, and died in the course of the 5-day experiment. Therefore, these animals did not take part in the open-field experiment. In the KYN+PROB+PTZ-treated group, the animals did not exhibit the development of any seizures in the course of the 5-day experiments and the mortality of these animals was very limited (Fig. 9).

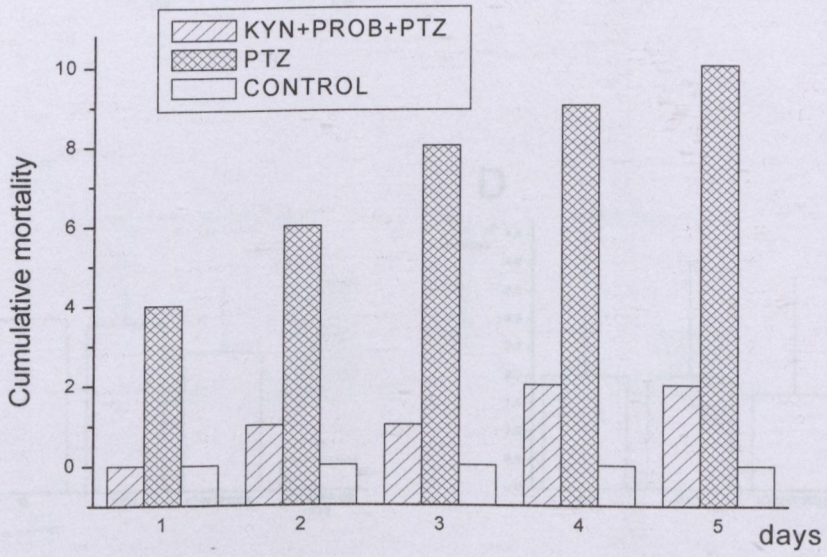

Figure 9. Cumulative number of dead animals in the groups of controls, PTZ-treated and KYN+PROB pretreated, then PTZ-injected animals during the 5-day experiment. Chronically administered PTZ (60 mg/kg, i.p.) resulted in the death of all the animals in the PTZ-treated group during 5 days. With the exception of two animals, KYN+PROB pretreatment protected the rats from the death otherwise induced by the repeated PTZ injections.

The animals in the KYN-, PROB-, KYN+PROB- and KYN+PROB+PTZ-treated groups did not display any significant difference between staying in the centre or at the periphery of the

arena: all animals preferred to stay at the periphery (Fig. 10A). Differences were observed in rearing, in stereotyped washing and in defecation activity between the controls and the other treated animals, but the differences in defecation activity were not significant (Fig. 10B,C,D).

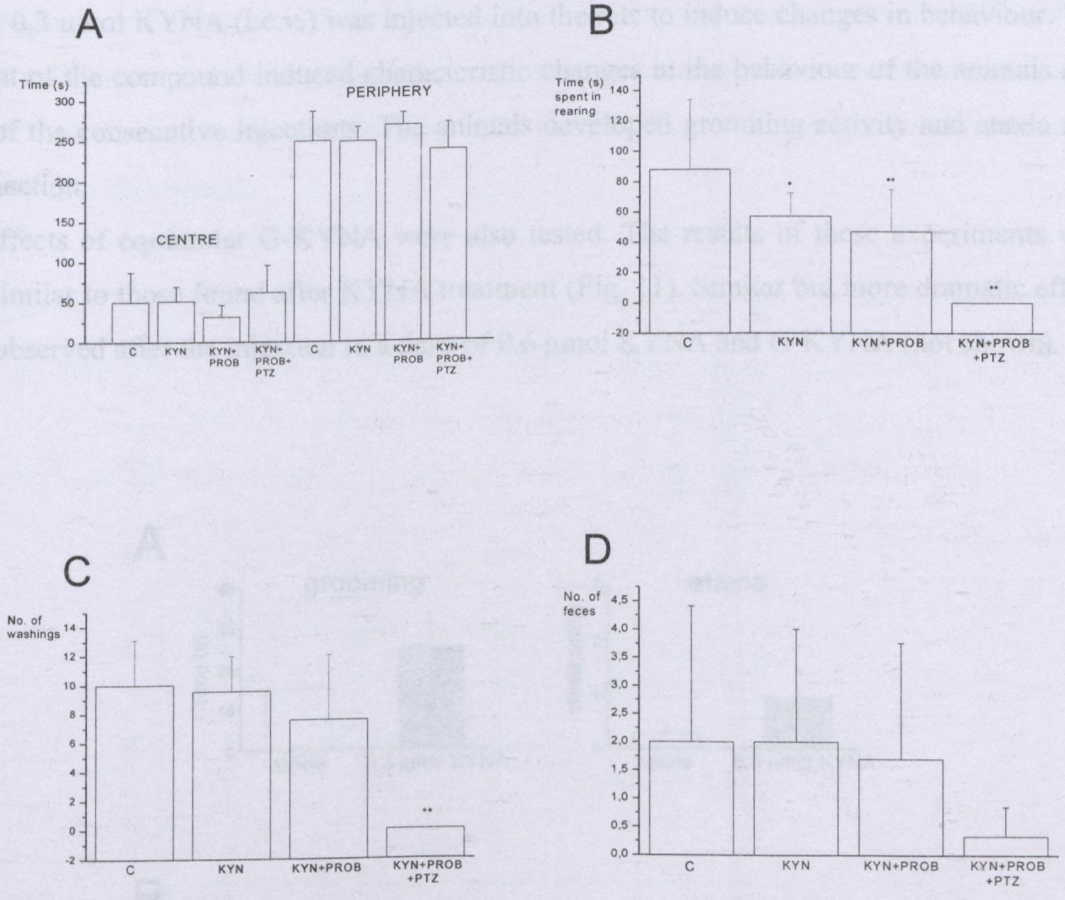

Figure 10. Observations in an open field arena. A: Time (s) spent in the centre and at the periphery of the arena. No difference was found between the control and treated groups. The rats in all four groups preferred to stay at the periphery of the arena. B: Time (s) spent in rearing during the 5-min observation period. The animals after KYN, KYN+PROB or KYN+PROB+PTZ injection became calm and slowed down. C: the number of washings was decreased significantly only in the KYN+PROB+PTZ-treated group during the 5-min observation. D: Number of faeces produced during the 5-min period. Data are shown as means ± s.d. *p<0.05, **p<0.01.

2.1.2. Open-field observations after KYNA and G-KYNA treatment

The behavioral effects of i.c.v. injected KYNA have already been well documented (Vecsei and Beal, 1990b; Vecsei and Beal, 1990a; Vecsei and Beal, 1991). In our experiments, we focused only on whether i.c.v. applied KYNA induced similar changes in behaviour under the present circumstances in order to make a comparison between the behaviour effects of these drugs. 0.3 μmol KYNA (i.c.v.) was injected into the rats to induce changes in behaviour. This amount of the compound induced characteristic changes in the behaviour of the animals after each of the consecutive injections. The animals developed grooming activity and ataxia after the injection.

The effects of equimolar G-KYNA were also tested. The results of these experiments were very similar to those found after KYNA treatment (Fig. 11). Similar but more dramatic effects were observed after the injection in a dose of 0.6 μmol KYNA and G-KYNA (not shown).

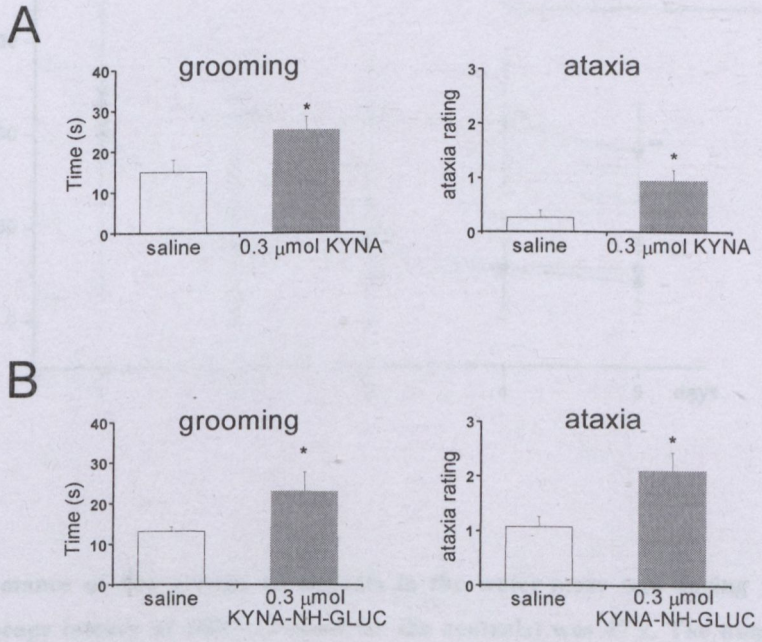

Figure 11. Effects of i.c.v. administered 0.3 μmol KYNA and G-KYNA on grooming and ataxia in rats. The controls received the same volume (10 μl) of saline. The behaviour was examined between 10 and 15 min after drug administration. Both KYNA and G-KYNA resulted in the development of grooming and ataxia. *p<0.05.

2.2. Water-maze task

2.2.1. Water-maze performance after KYN, PROB and PTZ treatment

All five groups of animals (KYN, PROB, PTZ, KYN+PROB and KYN+PROB+PTZ) started to take part in the water-maze experiments which began 20 min after the treatments. From the very beginning, the performance throughout the 5 days revealed that the KYN+PROB+PTZ-treated animals needed more time to reach the platform than did the controls. This tendency became marked from the sessions on day 2 and the difference then remained significant up to the end of the experiment.

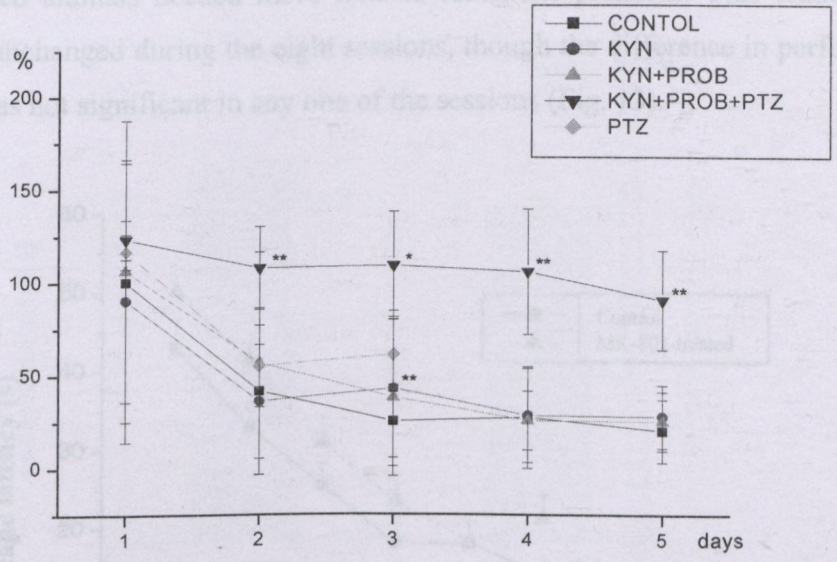

Figure 12. Performance of five groups of animals in the water-maze test during the 5-day period (4 trials/day). The escape latency at 100% (=mean for the controls) was 47 s. The number of PTZ-treated animals fell to 2 by day 4, and the evaluation of this group was therefore interrupted. *p<0.05, **p<0.01. Remark: On day 3, p<0.01 (control vs. KYNA+PROB+PTZ) and p<0.05 (control vs. PTZ).

The swimming strategies of the KYN+PROB+PTZ-treated animals and those in the other groups were not the same: the rats in the KYN+PROB+PTZ group spent more time swimming round the pool, near the wall, and demonstrated a poor performance throughout the 5-day trial. In contrast, the control animals displayed good progress in finding the hidden platform. Similarly, the animals in the KYN and KYN+PROB groups exhibited a good performance in the water-maze study. The PTZ-treated animals also started to reveal a good performance, but because of seizures and their high mortality rate, their testing was interrupted on day 3 (Fig. 12).

2.2.2. Water-maze performance after MK-801 treatment

From the very beginning, the performance during the eight training sessions revealed that the MK-801-treated animals needed more time to reach the platform. This tendency remained more or less unchanged during the eight sessions, though the difference in performance of the two groups was not significant in any one of the sessions (Fig. 13).

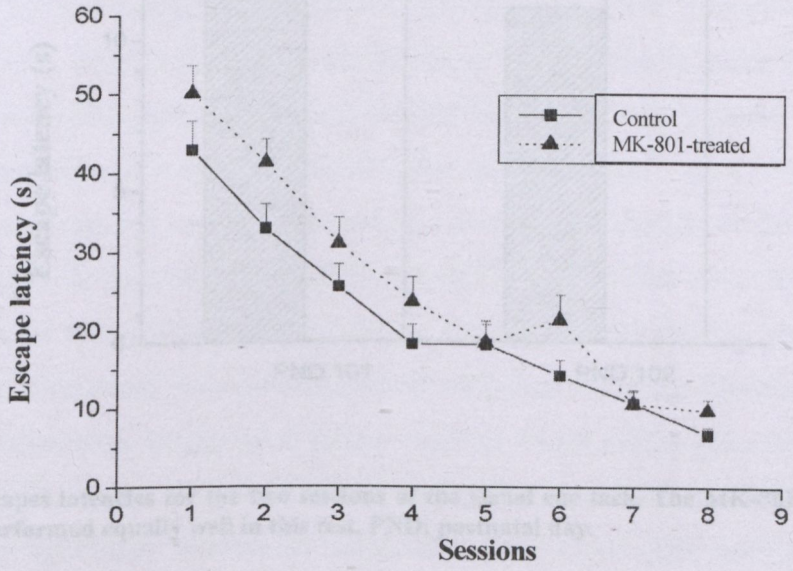

Figure 13. Escape latencies (in seconds) of adult controls and MK-801-treated animals. The performance of the MK-801-treated rats (0.1 mg/kg, twice daily, PND 7-19) was slightly poorer than that of the saline-treated controls.

It was interesting to observe that in the first trial in the first session the two groups needed approximately the same time to reach the platform. Later, the difference between the performances became larger, but did not attain the level of significance.
The swimming strategies of the treated and control animals were not the same: the treated animals spent more time swimming round the pool, by the wall. The difference in performance, however, was not due to a difference in anxiety or swimming speed between the two groups. Taken together, the MK-801-treated rats were capable of learning in the course of the sessions, but their performance tended to be poorer than that of the controls.

To check the possibility of modification of the sensory and motor capabilities by MK-801 treatment, a visual cue task was given to the animals in both groups. The visual cue task was performed equally well by both the controls and the MK-801-treated animals (Fig. 14).

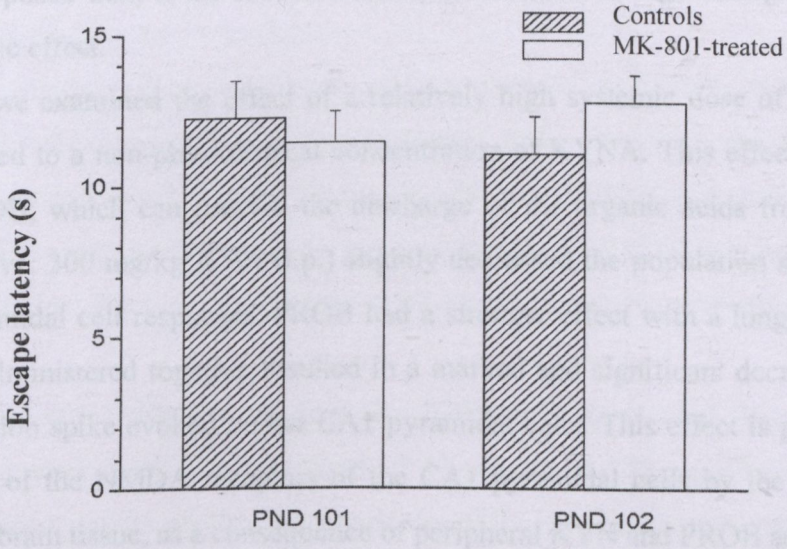

Figure 14. Escapes latencies for the two sessions of the visual cue task. The MK-801-treated animals and the controls performed equally well in this test. PND: postnatal day.

On the basis of the water-maze analysis, it can be stated in general that the performances of the MK-801-treated rats in the spatial learning and memory task were slightly (but not significantly) poorer than those of the controls. However, the visual cue task was performed equally well by both the controls and the treated animals.

Discussion

The normal concentration of KYNA in the brain is probably too low to influence the EAA receptors, and even under pathological conditions the data do not indicate that the concentration elevation will necessarily allow KYNA to influence the co-agonist site of the NMDA receptor. However, recent results reveal that the KYNA-sensitive presynaptic nACh receptors inhibit glutamate release at low concentration (30-100 nM) (Carpenedo *et al.*, 2001). It may therefore be speculated that these nACh receptors contribute to the inhibitory effects of KYNA at low concentration.

In 1984, Schwarcz's laboratory found that KYNA blocked both the neurodegeneration and the seizures caused by the local application of QUIN in the hippocampus and striatum (Foster *et al.*, 1984) and this inhibition was seen in behavioral tests, too (Wirsching *et al.*, 1989; Lekieffre *et al.*, 1990).

It may be supposed that, if the KYNA content of the brain is high enough, it has a definite neuroprotective effect.

First, we examined the effect of a relatively high systemic dose of KYNA precursor KYN which led to a non-physiological concentration of KYNA. This effect may be elevated by using PROB, which can prevent the discharge of the organic acids from the CSF. This proved effective: 300 mg/kg KYN (i.p.) slightly decreased the population spike amplitude of the CA1 pyramidal cell responses. PROB had a stronger effect with a longer delay. The two compounds administered together resulted in a marked and significant decrease in amplitude of the population spike evoked on the CA1 pyramidal cells. This effect is probably based on the inhibition of the NMDA receptors of the CA1 pyramidal cells by the elevated level of KYNA in the brain tissue, as a consequence of peripheral KYN and PROB administration.

PTZ is often used experimentally to induce seizures which mimic generalized clonic seizures in humans (Andre *et al.*, 1998). Indeed, 60 mg/kg PTZ administered i.p. to anaesthetized rats resulted in a significant increase in amplitude of the CA1 spike activity. *This effect was completely compensated by pretreatment with KYN+PROB.*

In parallel with the electrophysiological results, we studied the behavioral effects of KYN administered together with PROB 2 h before the PTZ injection. This convulsive dose of PTZ (60 mg/kg) caused epileptic seizures and death in awake animals. *It was found that*

KYN+PROB pretreatment completely protects awake rats from PTZ-induced clonic-tonic seizures and death. Although they exhibited reduced rearing, washing and defecation activity, we did not observe any difference in behavior between the controls and the treated animals in an open-field arena, though they revealed a significantly impaired performance in the water-maze task.

KYNA is an EAA receptor antagonist which can partially act at both the α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid and NMDA subunits of glutamate receptors (Swartz *et al.*, 1990; Stone, 1993). The overexcitation of these EAA receptors can lead to a high Ca^{2+} concentration and pathological processes, and finally start the apoptotic pathway in the cells. KYNA in high concentration is an effective neuroprotective agent, but its use is rather restricted because it has only a very limited ability to cross the BBB.

A new KYNA derivative, G-KYNA, was synthetized in the Institute of Medical Chemistry, University of Szeged. We presumed that this can readily cross the BBB and provide an elevated KYNA level in the brain tissue.

To check the similar effects of KYNA and its analogue G-KYNA, we tested these two compounds in behavioral and electrophysiological tasks. In agreement with the literature findings (Vecsei and Beal, 1990b; Vecsei and Beal, 1990a; Vecsei and Beal, 1991), the behavioral experiments revealed that *i.c.v. microinjected KYNA induces stereotype behaviour and ataxia. Similar changes in behaviour were found after i.c.v. administered equimolar G-KYNA.*

To examine whether G-KYNA can pass the BBB, we administered this drug i.v. and i.p. KYNA administered systemically in doses of 17, 34, 68 or 136 μmol/kg did not cause any observable change in the electrophysiological activity of the hippocampal CA1 region in anaesthetized animals. Against this, G-KYNA in a dose of 136 μmol/kg resulted in the death of the animals in all cases, while a dose of 68 μmol/kg (i.v.) induced the stoppage of breathing of the animals, though they could be resuscitated. *A dose of G-KYNA as small as 17 μmol/kg was effective in reducing the CA3 stimulation-evoked activity of the CA1 pyramidal cells in the hippocampus. This effect was augmented when G-KYNA was administered together with PROB.* PROB (200 mg/kg, i.p.) itself induced an increase in the brain KYNA content which is probably based only on the endogenous KYNA. A slight decrease in evoked activity was

observed with a long delay (1-1.5 h) in the experiments in which PROB or KYNA+PROB was injected i.p. into the rats.

NMDA receptors play an important role in spatial learning and working memory (Vianna *et al.*, 2000; Baker and Kim, 2002; Sargolini *et al.*, 2003). The perinatal blockade of these receptors might have behavioral consequences when tests are made at a more advanced age (Gorter and de Bruin, 1992). In the course of our working, we tested the hypothesis that even a minimal conflict with NMDA receptors in the early critical age of life, which results in mild detectable change in daily behaviour, induces hidden but life-long dysfunctions that can be detected in different parts of the central nervous system. We chose a low dose of MK-801 (0.1 mg/kg), injecting twice a day on PNDs 7-19, which caused a slight, not significant impairment of the performance in the water-maze task and produced hyperactivity, but did not impair the performance of the visual cue response of the adult animals. Later, these animals were used in electrophysiological examinations.

An increasing number of results suggest that transiently reduced inhibition (e.g. after nerve injury) is a necessary, but not sufficient condition for the development of MI plasticity. The potential for plasticity in the MI has been closely linked to the function of NMDA receptors (Qiu *et al.*, 1990). Moreover, it has been shown that a component of the field potentials evoked in the horizontal pathways of the rat motor cortex is mediated by these receptors and LTP can develop in these horizontal connections (Hess and Donoghue, 1994; Hess *et al.*, 1994).

Simple recordings of evoked potentials in the MI did not reveal significant differences between the controls and the MK-801-treated animals. Therefore, we tested a more complex phenomenon. The plasticity of evoked responses induced by facial nerve injury can be tested by the paired pulse paradigm (Toldi *et al.*, 1999; Farkas *et al.*, 2000). In the control rats, the responses evoked in the MIs of both hemispheres by continuous 1-Hz trigeminal stimulation were facilitated after facial nerve transection. *This was not the case with the MK-801-treated animals. In a majority of the cases studied, the evoked responses did not change, while in 40% of the cases, continuous stimulation reduced the evoked responses recorded after facial nerve injury.*

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